Transformed and Nontransformed Human T Lymphocytes Migrate to Skin in a Chimeric Human Skin/SCID Mouse Model

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To study human T cell migration to human skin *in vivo*, we grafted severe combined immunodeficient mice with 500-μm thick human skin. Two weeks after grafting, epidermal and dermal structures in the grafts were of human origin. When we intraperitoneally injected grafted mice with clones of the human HUT-78 T cell line derived from a patient with cutaneous T cell lymphoma and Sézary syndrome, we detected in the grafts the rare Vβ23-Jβ1.2 T cell receptor transcripts characteristic for the HUT-78 clones. These signals were found 2–6 d after cell injection in about 40% of the grafted and HUT-78 cell injected mice but not in grafts from mice that received no exogenous T cells. In contrast to HUT-78 cells, which only accumulate in low number, grafts topically challenged with nickel sufate in vaseline from mice that were injected with autologous nickel-reactive T cell lines led to massive accumulation of T cells within 3 d. Only scattered T cells accumulated in the skin when grafted mice received vaseline plus T cells, nickel sulfate alone, T cells alone, or nickel sulfate plus an allogeneic nickel-nonreactive T cell clone. When the T cell lines were labeled with the fluorochrome PKH-26 before cell injection, spots of fluorescent label in the size and shape of cells were found in the grafts challenged with nickel. Together, these results clearly demonstrate that human T cells can migrate to human skin in this chimeric human/mouse model. *Key words: cutaneous lymphocyte-associated antigen/skin transplantation/T cell receptor rearrangement/tumor necrosis factor-α. J Invest Dermatol 109:744–750, 1997*

Large numbers of skin diseases are associated with lesional infiltration by lymphocytes that are thought to induce skin inflammation. As healthy skin contains very few lymphocytes and only a few lymphocytes proliferate in inflamed skin, most infiltrating lymphocytes appear to originate from the circulation. In the last couple of years, many investigators have focussed on the mechanisms of leukocyte migration into tissue. *In vivo* studies in experimental animals such as mice have shown the involvement of several adhesion molecules and cytokines; however, these results may not always apply for humans in which mostly only correlative data were available (Brigham and Meyrick, 1986; Tryka *et al*, 1986). This situation demonstrated the need for new approaches to obtain functional information in humans. Albelda's group was the first to report a new approach to study leukocyte migration into human skin (Yan *et al*, 1993). They used human skin grafted to severe combined immunodeficient (SCID) mice that are unable to arrange T and B cell receptors (Bosma *et al*, 1983). With this model, they demonstrated the migration of murine and human neutrophils into human skin grafts (Yan *et al*, 1993; Murray *et al*, 1994).

We also used the human skin/SCID mouse model to study the migration of human T cells to human skin. Our preliminary studies, however, revealed that human skin grafts contained mononuclear cells, among them T cells. We also made observations that suggested that the number of mononuclear cells, including T cells, increased proportionally with the interval between grafting and graft analysis. Therefore, the mere presence or increase in mononuclear cells in such a model may not automatically indicate migration because local expansion of mononuclear cells including T cells may be the explanation. For these reasons, we first wanted to investigate if exogenously administered T cells can be identified in the grafts. To answer this question we used the human T cell line HUT-78 and a reverse transcriptase polymerase chain reaction (RT-PCR)-based method to identify the rare Vβ23-Jβ1.2 rearrangement of the T cell receptor (TCR)-β chain expressed by this cell line (Pannetier *et al*, 1995). We chose the HUT-78 cell line because it was derived from a patient with a T cell lymphoma involving the skin and with Sézary syndrome, reasoning that this cell line may have retained some capacity to migrate to human skin (Gootenberg *et al*, 1981). We show here that Vβ23-Jβ1.2 TCR transcripts found in HUT-78 cells can be identified in human skin grafted to SCID mice that have received HUT-78 cells intraperitoneally (i.p.), but the migration of these transformed T cells to the skin graft appeared to be limited. We therefore injected labeled nontransformed nickel-reactive T cells that were generated from allergic donors into SCID mice with autologous skin grafts. We identified labeled cells in human grafts only after activating the grafts with nickel.

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Abbreviations: CLA, cutaneous lymphocyte-associated antigen; i.p., intraperitoneally; LFA-1, leukocyte function-associated antigen-1; PE, phycoerythrin; RT-PCR, reverse transcriptase polymerase chain reaction; SCID, severe combined immunodeficiency; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

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Our results suggest that the human skin/SCID mouse model can be used for studying the migration of human T cells to human skin if injected T cells can be distinguished from the graft's resident T cells.

MATERIALS AND METHODS

Antibodies Rabbit anti-asialo GM1 was purchased from Wako Chemicals (Richmond, VA). The following antibodies were used for cytofluorometric analysis: monoclonal antibody (MoAb) HECA-452 against cutaneous lymphocyte-associated antigen (CLA) and a rat IgM isotype control (kindly provided by Dr. L. J. Picker, University of Texas Southwestern Medical School, Dallas, TX) were produced and used as described (Picker *et al*, 1990), MoAb phycoerythrin (PE)-conjugated mouse anti-human CD4 was purchased from Becton Dickinson (Mountain View, CA), MoAb fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD11a and mouse anti-human CD71 were purchased from Pharmingen (San Diego, CA), MoAb mouse anti-human CD49d (VLA-4) was purchased from Immunotech (Marseille, France). The mouse anti-human IL-2R (anti-TAC), mouse anti-human HLA-DR (clone L243), mouse anti-human CD8 (clone OKT8), mouse isotype controls anti-mouse Vβ 8.1–3 (clone F23.1), and anti-trinitrophenyl (clone 1B7.11) were used as supernatants from hybridomas purchased from the American Type Culture Collection (ATCC, Rockville, MD). Goat anti-rat (H + L) coupled to FITC was from Immunotech and rat anti-mouse coupled to PE was from Becton Dickinson. The following MoAb were used for immunohistology: mouse anti-human major histocompatibility complex (MHC) I (W6/32), mouse anti-human MHC II (HB55), and rat anti-mouse MHC II (M5) were used as supernatants from hybridomas purchased from ATCC. Mouse anti-human ICAM-1 (clone E1/DR) was kindly provided by S. Carrel (Ludwig Institut, Lausanne, Switzerland), mouse anti-human CD1a (clone OKT6), mouse anti-human CD31, and rabbit anti-human CD3 were from Dako (Glostrup, Denmark), mouse anti-human vascular cell adhesion molecule-1 (VCAM-1) and mouse anti-human E-selectin were from Vector Laboratories (Burlingame, CA), and rat anti-mouse CD31 was from Pharmingen. Biotinylated horse anti-mouse and rabbit anti-rat were purchased from Vector Laboratories and swine anti-rabbit was from Dako.

Cell preparation The HUT-78 cell line is a HTLV-1$^+$ lymphoma cell line derived from a patient with cutaneous lymphoma and Sézary syndrome and was obtained from the ATCC (Gootenberg *et al*, 1981). CLA$^-$ and CLA$^+$ HUT-78 clones cultures were generated and cultivated as described (Santamaria Babi *et al*, 1996). Nickel-specific T cells were generated *in vitro* by stimulating mononuclear cells (1×10^6 cells per ml) isolated from peripheral blood of nickel allergic donors by centrifugation on Ficoll-Paque gradients, with 5 μg nickel sulfate per ml and recombinant human interleukin-2 (50 U per ml, Eurocetus, Amsterdam, The Netherlands). T cells were restimulated every 2–3 wk with nickel sulfate and autologous B cells immortalized with Epstein–Barr virus as described (Tang *et al*, 1993). The specificity of the cell lines was confirmed in standard proliferation assays. The lines were used after 2–3 restimulation cycles. A nickel-nonreactive T cell clone was derived from peripheral blood using the "PHA cloning" technique (Sinigaglia *et al*, 1985). Its phenotype was 100% CD4$^+$, 70% CLA$^+$.

Five $\times 10^5$ cells were incubated at 4°C for 30 min with the first antibody. Then, the cells were washed twice in phosphate-buffered saline (PBS) containing 1 mg bovine serum albumin per ml and 0.1% NaN_3 and incubated with an adequate second anti-mouse or anti-rat antibody coupled to FITC or PE. Stained cells were fixed in 0.5% paraformaldehyde and stored at 4°C until analyzed on a FACScan^R flow cytometer (Becton Dickinson).

PKH-26 GL kit (Sigma, St. Louis, MO) was used to stain the HUT-78 and nickel-specific T cells before injection according to the manufacturer's instructions. We confirmed by fluorescence-activated cell sorter analysis that 99% of the cells were strongly labeled and by trypan blue exclusion that the stained cells were viable. The migration of the labeled cells was assessed on 7-μm frozen sections using a fluorescence microscope with a filter for PE detection. Four consecutive sections every 140 μm were analyzed.

Histology and immunohistology Human graft samples fixed in Dubosq-Brazil were used to prepare paraffin-embedded sections stained with hematoxylin and eosin. Paraffin-embedded sections were also used to detect human CD3$^+$ cells. Five-μm cryostat sections were prepared from human grafts. Three consecutive sections every 50 μm were analyzed. Prior to incubation for 1 h with the first MoAb, sections were fixed in acetone and washed in PBS containing 1% bovine serum albumin and 0.2% Tween. Bound antibody was detected with a commercially available kit of secondary antibodies coupled to immunoperoxidase (Vector Laboratories); 3,3′ diaminobenzidine dihydrochloride (Sigma) was used as a chromagen and the sections were counterstained with hemalaun. Controls were performed by incubating sections with PBS instead of the first MoAb.

Skin grafting Female CB17 SCID mice were purchased from Iffa Credo (Arbresle, France) and used at 6–8 wk of age. The mice were kept in isolators under barrier-sustained conditions with provisions of sterilized water, food, and bedding. Human buttock skin was obtained from healthy or nickel-allergic volunteers (age 27–50 y) with permission from the local ethics committee. Sheets of 500-μm thick skin were harvested with a dermatome knife. Skin sheets were kept in ice-cold PBS and grafted within 2 h. CB17 SCID mice were anesthetized by intraperitoneal injection of xylozine (Bayer, Germany, 3 mg per kg) and ketamine (Rhône Merieux, France, 3 mg per kg). The mice were shaved and one 1.5 × 1 cm skin segment was excised from their back. The wound was immediately covered with the human skin. The transplant was held in place with 6–0 nonabsorbable monofilament suture material and covered with vaseline and adhesive tape. The dressing material and sutures were removed after 7–10 d. The dorsal location of the grafts prevented the mice from being able to scratch the grafts. Each mouse was housed separately.

Injection of antibodies, cytokines, and cells Two weeks after grafting, the mice with grafts that did not show signs of necrosis were pretreated by intravenous injection of 50 μl anti-asialo GM1 antibody. Twelve hours later, grafts were injected or not with 2000 U of recombinant human TNF-α (Genzyme) diluted in 50 μl of PBS containing 2 mg bovine serum albumin (Sigma, Buchs, Switzerland) per ml and 5% Evans blue, to mark the site of injection. Another 12 h later, mice received HUT-78 cell clones (150×10^6 cells per mouse) intraperitoneally. The grafts of other mice injected with antibody 12 h previously received vaseline or 2% nickel sufate under occlusion. Twelve hours later the mice received nickel-reactive T cells ($10–15 \times 10^6$ cells per mouse) intraperitoneally. The graft skin of these mice originated from the same donor as the nickel-reactive T cells. Mice were sacrificed and the grafts removed and divided into three pieces; one was placed in Dubosq-Brazil and the other two were snap frozen in liquid nitrogen. Fragments from various mouse organs were also snap frozen and stored in liquid nitrogen.

RNA extraction and cDNA synthesis Human skin grafts or adjacent murine skin was cut into 8-μm thick sections with a cryostat, dissolved in 1 ml of Trizol (Gibco, Basel, Switzerland), and stored at −80°C until total RNA extraction. The other organs were pulverized in a mortar under liquid nitrogen, and the powdered tissue was transferred into 1 ml of Trizol. RNA was extracted from skin, other tissue samples, and HUT-78 cells according to standard procedure (Chomczynski and Sacchi, 1987). The first-strand of cDNA was synthesized in a 50-μl final volume at 37°C for 1 h using 5 μg of total RNA, 200 U MMLV (moloney murine leukemia virus) reverse transcriptase (Gibco), and 40 U RNase (Promega, Madison, USA). The reaction was stopped by heating for 5 min at 95°C and then stored at −20°C until PCR amplification.

TCR-β chain rearrangement of the HUT-78 clones and its identification in tissue samples The specificity of a given T cell is determined by the expression of unique rearranged TCR gene products, and in particular by the complementarity determining region 3 (CDR3) of both α and β chains. This region corresponds to the hypervariable region of immunoglobulin molecules and is essential for the binding of the TCR to antigenic peptides presented by MHC molecules. Thus, we used a recently developed RT-PCR-based method for the determination of transcript length variability of the TCR-β chain CDR3 region (Pannetier *et al*, 1995). Experiments were performed as previously described (Dietrich *et al*, 1994; Pannetier *et al*, 1995). Briefly, the cDNA of both HUT-78 clones was amplified using a panel of experimentally validated 5′ sense primers specific for the 24 Vβ subfamilies and one 3′ anti-sense primer for the Cβ gene segment (Genevee *et al*, 1992). As the CLA$^+$ and CLA$^-$ HUT-78 clones exclusively expressed Vβ23 transcripts, their clonality was further defined by subjecting Vβ23-Cβ PCR products to one cycle of elongation using dye-labeled oligonucleotide primers specific for the 13 human Jβ segments. The primers labeled with a 6-fam fluorophore were purchased from Genset (Paris, France). The identification of the Vβ23-Jβ1.2 rearrangement (size of 232 nt) found in HUT-78 cells was achieved using the corresponding Vβ23 and Jβ1.2 primers. The run-off products were then run on an automated sequencer (Applied Biosystems, Perkin Elmer, Foster City, CA) in the presence of fluorescent size markers. The length of the DNA fragments and the fluorescent intensity of the bands were analyzed with the Gene Scan Analysis software (Applied Biosystems). Negative control was performed without cDNA. As a control, we also examined the presence of Vβ13 transcripts in all samples using the same procedure.

RESULTS

Grafted skin contains human cells To establish an *in vivo* model for the migration of human T cells to the skin, we first transplanted human skin onto SCID mice. After 2 wk, the grafts showed signs of viability. Histologic analysis revealed an orthokeratotic stratified

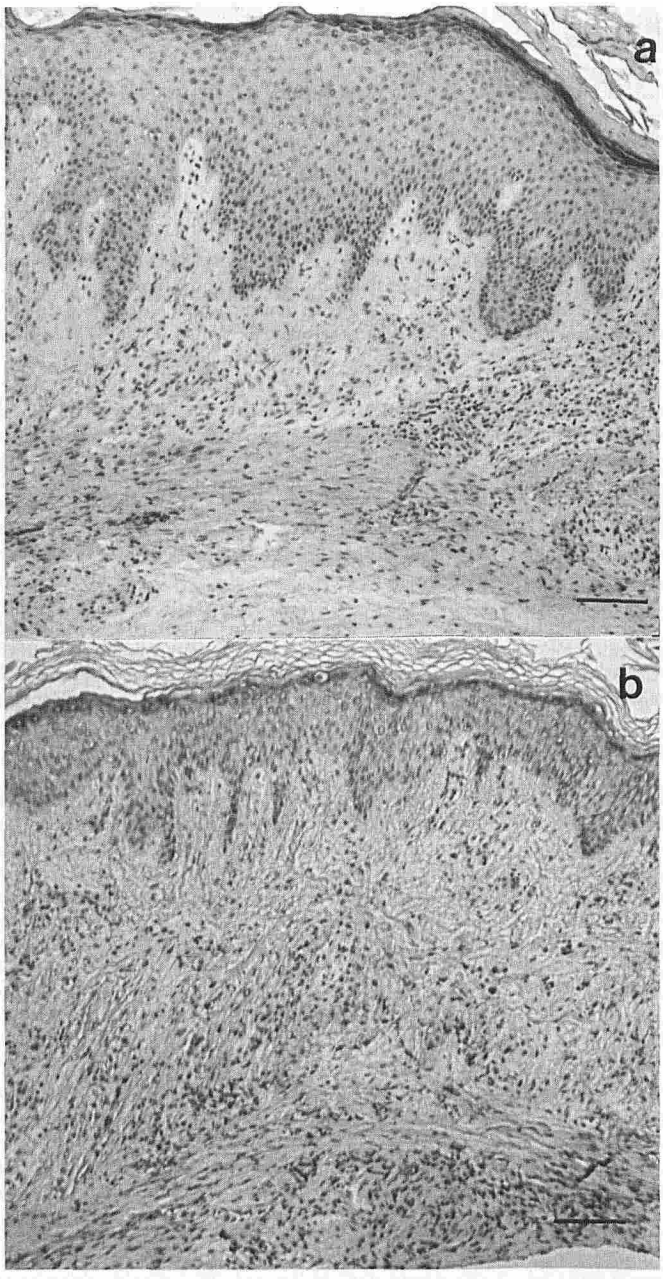

Figure 1. Mononuclear cells in the unmanipulated grafts of mice that received CLA$^+$ HUT-78 cell clones. Sections of human skin grafts from SCID mice that (*a*) did or (*b*) did not receive CLA$^+$ HUT-78 cell clones 4 d previously. *Hematoxylin and eosin; scale bars, 100 μm.*

epithelium with slight acanthosis and a preserved superficial dermal structure. Below the superficial epidermis, fresh granulation tissue and, in some specimens, the murine musculus carnosus could be distinguished. The superficial dermis contained a moderate and diffuse cell infiltrate composed of presumably murine polymorphonuclear leukocytes and of mononuclear cells. The number of fibroblastic cells seemed to be slightly increased (**Fig 1*b***). The species-specific cellular composition of the grafts was studied by immunohistochemistry. In all of the grafts tested (n = 12), epidermis and dermis was of human origin, as indicated by staining with the anti-human MHC class I MoAb W6/32. In all human skin grafts tested, we detected human CD1a$^+$ cells, presumably Langerhans cells, diffusely spread throughout the epidermis, unlike in normal human skin *in situ*. A similar pattern was detected for human MHC class II$^+$ cells in the dermal and epidermal compartments. Furthermore, human CD31$^+$ endothelial cells could be clearly distinguished in a cord pattern in the dermis, whereas no positive cells for the human adhesion molecules ICAM-1, VCAM-1, or E-selectin were detected. Human CD3$^+$ T cells were observed in the dermis of the human skin grafts in numbers clearly greater than in pregraft skin. A few singular murine MHC class II$^+$ cells were detected in the grafted dermis.

Stimulation of grafts results in activation We speculated that the migration of the injected human T cells may be facilitated by an inflammatory stimulus. We thus injected human TNF-α into the skin grafts (nine mice). Four days after TNF-α injection, we detected no significant tissue alteration with the exception of an increase of cell number in the dermis of the grafts compared with noninjected grafts. This increase was due to mononuclear and polymorphonuclear cells in the dermis. The staining pattern of CD1a$^+$ cells and murine MHC class II$^+$ cells was not affected by TNF-α. We could distinguish an increased number of human MHC II$^+$ cells in the dermis comprising fibroblasts, mononuclear cells, and endothelial cells. In addition, human CD31$^+$ cells were more numerous in all TNF-α-treated grafts compared with noninjected grafts. No staining for the human ICAM-1, VCAM-1, and E-selectin was found 4 d after TNF-α injection. In contrast, these adhesion molecules could be identified in CD31$^+$ cells by immunostaining 8 h after TNF-α injection (four mice). In the histologic image there was no difference between noninjected and PBS-injected grafts (five mice).

Topical application of nickel sulfate in vaseline was tested at different concentrations in order to determine the optimal dose of nickel that will activate the grafts without inducing gross tissue destruction. Two per cent nickel sulfate induced the migration of a small number of murine cells without histologically detectable graft destruction (12 mice). Furthermore, the number of human MHC class II$^+$ cells and CD31$^+$ cells in the grafts was increased. No increase of murine MHC class II$^+$ cells was detected compared with vaseline-treated grafts. No staining for human ICAM-1, VCAM-1, and ELAM-1 was found 3 d after nickel application, whereas staining for these adhesion molecules was clearly distinguishable on presumably endothelial cells 8 and 10 h after nickel application (four mice).

Characterization of the injected cells We next searched for suitable cell sources for injection into grafted mice. As transformed T lymphocytes, we chose the human T cell line HUT-78 that was derived from a patient with a cutaneous T cell lymphoma and Sézary syndrome (Gootenberg *et al*, 1981), and used sublines that had either high levels of CLA expression (65–86%, three independent experiments) or did not stain for CLA (Santamaria Babi *et al*, 1996). CLA has previously been thought to play a role in the migration of T cells to the skin (Picker *et al*, 1990). As nontransformed T lymphocytes, we used nickel-specific T cell lines from nickel allergic donors.

To further characterize the HUT-78 clones, we determined their surface marker profile by flow cytometric analysis. All cells from the CLA$^+$ and CLA$^-$ HUT-78 sublines expressed HLA-DR, 60–80% of the cells from both sublines expressed CD71, 20–50% of the cells of both clones expressed CD3 and CD4, 1–5% of the cells from both clones were stained with CD25, but no significant staining of both clones was observed with MoAb to CD49d (VLA-4), CD11a (LFA-1) or CD8 (three independent experiments). The nickel-reactive T cell lines expressed the following markers at the time of injection (two independent experiments, five independent donors): CD3 [94 ± 2% (mean ± SEM)], CD4 (89 ± 7%), HLA-DR (41 ± 10%), LFA-1 (98 ± 1%), VLA-4 (18 ± 9%), CLA (11 ± 8%), CD25 (48 ± 13%), CD71 (8 ± 11%).

In order to distinguish HUT-78 cells from T cells in the graft, we determined the TCR-β chain rearrangement of both sublines. We found a unique Vβ23-Jβ1.2 gene rearrangement, the amplified fragment showing a 232 nucleotides size in both sublines. This Vβ-Jβ gene rearrangement is unusual in other T cells (Choi *et al*, 1989; Rosenberg *et al*, 1992), suggesting that we would have little chance to detect this specific β-chain rearrangement of this size in grafts from mice that did not receive HUT-78 clones. Contrary to the HUT-78 T cells, nickel-reactive T cells express a diverse TCR repertoire (data not shown).

Vβ23-Jβ1.2 transcripts can be observed in grafts after i.p. injection of HUT-78 clones The histologic and immunohistologic

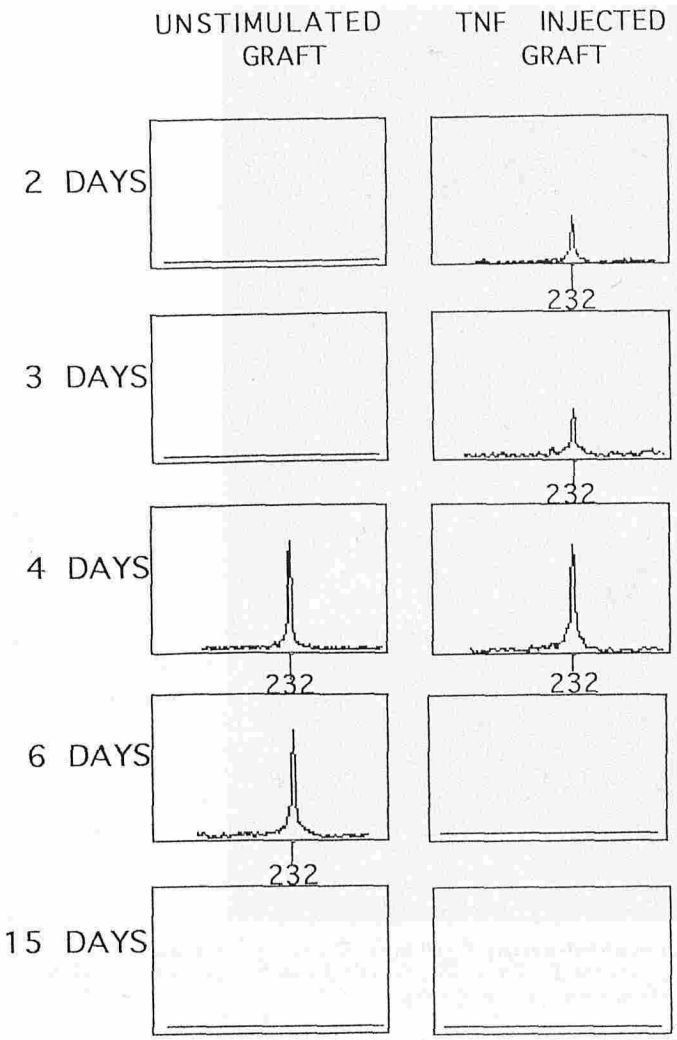

Figure 2. Vβ23-Jβ1.2 transcripts are detected in the grafts 2–6 d after intraperitoneal injection of CLA$^+$ HUT-78 cell clones. Extracts from biopsies of skin grafts stimulated (*right column*) or not stimulated (*left column*) by i.d. injection of TNF-α (2000 U) were prepared on the indicated days after i.p. administration of CLA$^+$ HUT-78 cell clones and subjected to RT-PCR with primers specific for Vβ23 and Jβ1.2 gene segments. The patterns show the size (*x*-axis) and intensity (*y*-axis) distribution of in-frame Vβ products according to their migration time; the expected transcript size of 232 nucleotides, corresponding to a CDR3 of nine amino acids, is indicated on the *x*-axis.

comparison of grafts from HUT-78-injected animals revealed merely a slightly denser human mononuclear cell infiltrate in the dermis than in the controls whether TNF-α was injected or not (**Fig 1*a*** and data not shown, respectively). No epidermotropism of the mononuclear cell infiltrate could be detected. Furthermore, we stained the HUT-78 cells with the PKH-26 fluorochrome before injection, but no fluorescent cells could be detected in the grafts 4 d after cell injection (data not shown).

We sacrificed mice on days 2, 3, 4, 6, and 15 and analyzed skin grafts (both TNF-α and noninjected) for the presence of Vβ23-Jβ1.2 transcripts. Vβ23-Jβ1.2$^+$ signals of the correct size (232 nucleotides) were detected in unmanipulated human grafts 4–6 d after injection of HUT-78 cells. TNF-α injection induced Vβ23-Jβ1.2$^+$ transcripts in the grafts 2–4 d after cell injection. No signal for Vβ23-Jβ1.2 in both conditions was detected between days 7 and 15 (**Fig 2**), suggesting that HUT-78 cells were not retained in the grafts. No Vβ23-Jβ1.2 signals were found in adjacent murine skin on days 2, 3, 4, 6, and 15. In accordance with this preliminary time-course experiment, we determined Vβ23-Jβ1.2 signals 4 d after cell injection in a larger panel of mice (**Fig 3**). Vβ23-Jβ1.2 signals were found in 43.5% of the grafts that received intracutaneous TNF-α, but in only 15.7% of grafts that

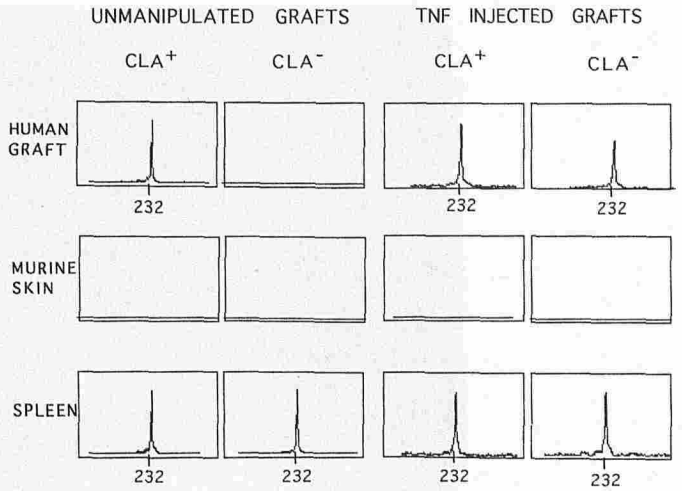

Figure 3. Vβ23-Jβ1.2 transcripts can be identified in human skin grafts and in the spleen of SCID mice but not in murine skin adjacent to the grafts. Vβ23-Jβ1.2 transcripts in biopsies of noninjected (unmanipulated grafts) or TNF-α-injected skin grafts (TNF-injected grafts) of mice that received either CLA$^+$ or CLA$^-$ clones generated from the HUT-78 cell line. The pattern shows the size (*x*-axis) and intensity (*y*-axis) distribution of in-frame Vβ products according to their migration time; the expected transcript size of 232 nucleotides, corresponding to a CDR3 of nine amino acids, is indicated on the *x*-axis. The result of one representative experiment is shown (see also **Table I** for compilation of results).

were not injected ($p = 0.04$, Fischer's exact test, **Table I**). Each RT-PCR reaction was repeated twice with the same qualitative results. When the grafts were not deliberately stimulated, signals for Vβ23-Jβ1.2 were found in three of 10 mice injected with CLA$^+$ HUT-78 cells but in none of 10 mice injected with CLA$^-$ HUT-78 cells ($p = 0.12$). No cells using the Vβ23-Jβ1.2 gene segment were detected in grafts of the 10 SCID mice that received PBS instead of HUT-78 cells (thirteen of 42 *versus* none of 10, $p = 0.01$), thus validating our experimental method for the identification of Vβ23-Jβ1.2$^+$ transcripts. Conversely, other Vβ signals such as the commonly expressed Vβ13 transcripts were present in these control grafts (data not shown), confirming the presence of resident T cells in the grafts.

We detected Vβ23-Jβ1.2 TCR-β chain transcripts also in murine organs such as spleen ($n = 13$), lung ($n = 13$), and thymus ($n = 6$) from grafted SCID mice 4 d after the injection of the HUT-78 clones (**Fig 3**). In contrast, no such transcripts were detected in murine skin ($n = 14$) adjacent to noninjected and TNF-α-injected grafts (grafts *versus* adjacent murine skin, $p = 0.025$, **Fig 3**), suggesting that CLA$^+$ and CLA$^-$ HUT-78 cells had a more favorable hosting environment in human than in murine skin.

Exogeneously administrated nickel-reactive T cell lines migrate into grafts challenged with nickel An abundant mononuclear dermal cell infiltrate was observed in nickel-challenged grafts ($n = 12$) of mice injected with nickel-reactive T cell lines that were autologous to the skin grafts (**Fig 4*a***). No spongiosis was detectable in any of the grafts but the epidermis was necrotic in seven of 12 (**Fig 4*a***). Application of nickel alone, vaseline alone, and administration of cells alone caused a sparse mononuclear cell infiltrate in the grafts and no epidermal necrosis (**Fig 4*b–d***). Furthermore, the injection of a nickel-nonspecific allogeneic human T cell clone in mice whose grafts were activated by nickel application led to sparse mononuclear cell infiltrate compared with nickel-challenged grafts of mice with nickel-specific T cells (data not shown). In comparison to all control grafts, we observed a high number of human MHC class II$^+$ and CD3$^+$ cells in the dermis of nickel-challenged grafts from mice that received nickel-reactive T cell lines. To investigate whether the injected T cells migrated to the grafts, we labeled the nickel-reactive T cell lines with the PKH-26 fluorochrome before injection. Three d after cell injection we detected many fluorescent dots in the size and shape of cells only in nickel-challenged grafts from mice that were injected with nickel-reactive T

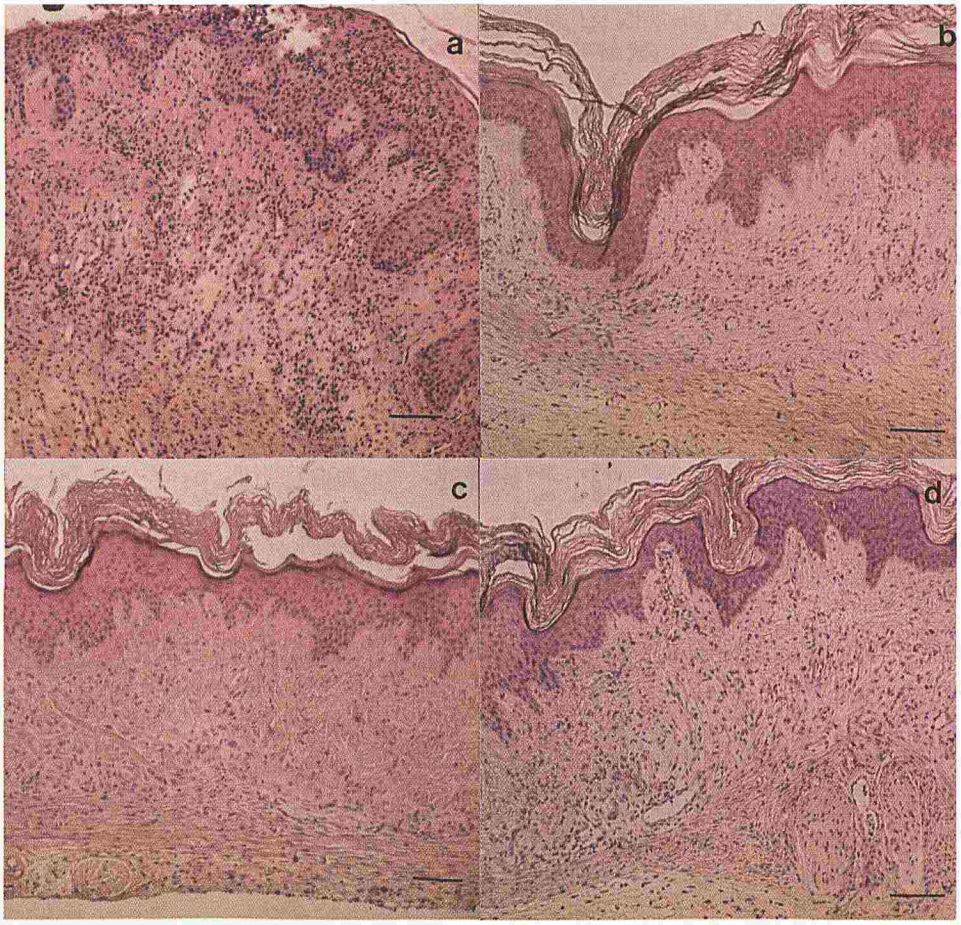

Figure 4. Graft alterations induced by topical nickel sulfate and i.p. injection of nickel-reactive T cell lines. Sections of human skin grafts from mice that received nickel-reactive human T cell lines (*a*, *b*, *d*). The grafts that were autologous to the T cell lines were pretreated with 2% nickel sulfate in vaseline (*a*, *c*) or vaseline alone (*d*). Graft biopsies were taken 3 d after T cell injection. Hematoxylin and eosin; *scale bars*, 100 μm.

Table I. Detection of Vβ23-Jβ1.2 transcripts in nonmanipulated or TNF-α-injected grafts from mice that received PBS, CLA^+, or CLA^- HUT-78 cell clones intraperitoneally[a]

Graft skin injected with	Mice injected i.p. with	Number of grafts	Number of graft donors	Number of $V\beta23J\beta1.2^+$ grafts	% of positive grafts[b]
- - -	CLA^+ cells	10	5	3	30
	CLA^- cells	9	5	0	0
	PBS	5	5	0	0
TNF	CLA^+ cells	13	5	6	46
	CLA^- cells	10	5	4	40
	PBS	5	5	0	0

[a]Nonmanipulated or TNF-α (2000U)-injected grafts from mice which received CLA^+, CLA^- HUT-78 cell clones, or PBS intraperitoneally 12 h later. The mice were sacrificed 4 d after cell injection. The Vβ23-Jβ1.2 rearrangement (expressed by both CLA^+ and CLA^- HUT-78 cells) was identified in graft biopsies RNA using RT-PCR and migration of fluorescent PCR products of an automated sequencer. $p = 0.04$ between TNF and noninjected recipients that received HUT-78 cells.

[b]The results were expressed as the percentage of grafts in which Vβ23-Jβ1.2 transcripts were detected.

cells (six mice; **Fig 5*a***). Fluorescent cells were mainly confined to the dermis (**Fig 5*a***) but labeled cells could also only rarely be identified in the epidermis. Grafts from mice that received vaseline plus labeled T cells (four mice; **Fig 5*b***) or grafts from mice that received labeled T cells alone (three mice) contained only scattered fluorescent dots. These results indicated that injected T cells migrated preferentially to the grafts when they were stimulated with nickel.

DISCUSSION

We studied the migration of human transformed and nontransformed T cells into skin by using a human skin/SCID mouse model. Two weeks after grafting of human skin to SCID mice, the epidermis and dermis were of human origin and included epidermal $CD1a^+$ cells, presumably Langerhans cells, human MHC class II^+ cells, and human $CD31^+$ endothelial cells in the dermis. It was particularly important to check for chimerism in the light of a report in which the epidermis of the grafts was of murine origin in a similar human skin/SCID mouse model (Boehncke *et al*, 1994). Two weeks after transplantation, we detected a sparse mixed human and murine mononuclear cell infiltrate in the dermis of the grafts when compared with ungrafted human skin. The human infiltrate consisted of MHC class II^+ cells and T cells. This infiltrate was much denser when grafts were analyzed later than 4 wk after grafting (data not shown), indicating that resident T cells expanded in the graft. For this reason it cannot formally be concluded from the work of Murray *et al* (1994), who injected allogeneic T cells into SCID mice with human skin grafts, that the T cells found in the dermis and epidermis originated from the allogeneic donor. This finding should also be kept in mind in light of results published by Petzelbauer *et al* (1996) and Kunstfeld *et al* (1997). Therefore, we searched for a reliable marker to identify injected T

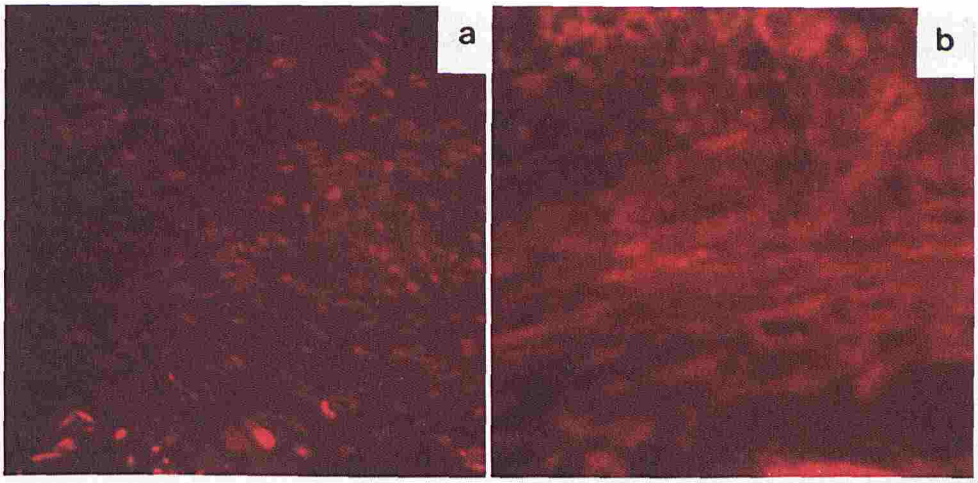

Figure 5. Accumulation of PKH-26 fluorescence in human skin grafts of mice that received autologous nickel-reactive T cell lines. Red (PKH-26) fluorescence of sections from (*a*) nickel sulfate in vaseline or (*b*) vaseline challenged grafts from SCID mice that received nickel-reactive T cell lines 3 d previously. Note the dot-like cellular labeling in (*b*) compared with the fibrillar background staining in (*a*). *Scale bars*, 50 μm.

cells in the grafts, and we used the animals 2 wk after grafting when the mononuclear infiltrate resulting from resident T cells was sparse.

As transformed T cells, we injected CLA^+ or CLA^- sublines generated from the HUT-78 cell line. Both sublines expressed the rare Vβ23-Jβ1.2 TCR gene rearrangement (Choi *et al*, 1989; Rosenberg *et al*, 1992). Charley *et al* have previously grafted human skin from a patient with cutaneous T cell lymphoma and Sézary syndrome onto SCID mice (Charley *et al*, 1990) but they did not show that i.p. or intravenously injected lymphoma cells can localize to the graft. Thanks to the RT-PCR run off methodology, we found this distinct Vβ23-Jβ1.2 mRNA in a fraction of grafts from mice that received HUT-78 clones but never in grafted mice that received no HUT-78 cells. With the exception of the remote possibility that non-HUT-78 T cells with the same TCR-Vβ chain rearrangement and transcript size reside in the skin graft and expand selectively *in situ* only when HUT-78 cells are injected, our results indicate that the i.p. injected HUT-78 cells arrived in the human skin grafts. Thus, this is the first *in vivo* demonstration that human T cells can home to human skin. The injection of TNF-α into the grafts led to an increase in the number of grafts in which we detected the Vβ23-Jβ1.2 transcripts. Given the biologic properties of TNF-α this suggests that this cytokine induces a favorable environment for the HUT-78 cells in the graft. This is reminiscent of the migration of human and mouse granulocytes into TNF-α, injected grafts using the same model as reported by Yan *et al* (1994). Recently, Veelken *et al* (1996) have reported that stimulation of skin with a patch test in patients with cutaneous T cell lymphoma may also lead to the detection of the malignant clone in previously normal-appearing skin, suggesting that local cytokine release in the skin can attract malignant T cells. The action of TNF-α includes the upregulation of the human E-selectin, VCAM-1, and ICAM-1 adhesion molecules (Yan *et al*, 1993), but the HUT-78 cells did not express detectable VLA-4 or LFA-1, the counter-receptors for VCAM-1 and ICAM-1, respectively. Thus, the mechanisms by which TNF-α induced the localization of HUT-78 T cells in the grafts remain to be elucidated. But TNF-α induces many other cytokines that in turn could contribute to the migration of the HUT-78 cells to the grafts. That TNF-α injection in our model had complex effects is supported by the observation of MHC class II induction in the grafts.

The presence of T cells in the extravascular space of the skin depends on many factors. T cells may enter, remain in the skin, undergo necrosis or apoptosis, or leave the skin. The sum of all these processes will determine if injected T cells can be identified in the skin at a given time point. Obviously, the conditions were united for Vβ23-Jβ1.2 transcript detection in only a fraction of our human skin/SCID mice; however, our skin grafts have the capacity to accumulate T cells in relatively large numbers. When we used the PKH-26 dye in nontransformed nickel-specific T cells a massive accumulation of labeled T cells in the nickel-activated grafts 3 d after T cell injection was found. When this dye was used to follow the HUT-78 cells to the skin we did not detect labeled cells 4 d after cell injection. When PKH-26-labeled cells were kept in culture for 4 d, their label was, however, very faint. Thus, the PKH-26 dye may have been too much diluted for detection in skin sections 4 d after cell administration. Furthermore, the number of HUT-78 cells in the grafts may have been too sparse to be detectable by fluorescence microscopy.

It is possible that the presence of HUT-78 cells in the grafts varied considerably from one animal to the other. Such a variability may explain the low relative number of grafts in which we identified the Vβ23-Jβ1.2 signal. Kinetic variability may also account for the difference between TNF-α-injected and noninjected grafts but it was impossible to do the time-course experiment with several mice per time point. It is also possible that the efficiency of HUT-78 cells to migrate or to localize into human skin is limited compared with nickel-reactive T cells. Such a limited capacity to migrate to human skin could be monitored by comparing simultaneously the presence of Vβ23-Jβ1.2 signals in blood and skin. Thus, the conclusions that can be drawn from the experiments with HUT-78 cells may not be relevant for the physiology of skin migration by nontransformed T cells.

We tried to investigate the role of the CLA in the migration of the T cells to human skin with the CLA^+ and CLA^- HUT-78 clones. CLA has been suspected to be a "skin-homing" receptor for T cells (Picker *et al*, 1990). CLA expressed on one of the HUT-78 clones that we used did not significantly augment the detection of Vβ23-Jβ1.2 transcripts in the grafts, although we have previously shown that the presence of CLA on the same HUT-78 clones clearly enhanced their migration across activated skin-derived endothelial cells in an *in vitro* assay (Santamaria Babi *et al*, 1995). These results suggest that the HUT-78 cells are not suitable to support the putative skin homing role of CLA in the human skin/SCID mouse model or that the expression of the CLA receptor alone was not able to induce a cell migration to skin grafts as discussed elsewhere (Kunstfeld *et al*, 1997). These authors have shown in a similar model using allogeneic T cells that CLA expression in perivascular T cells correlated with the superficial but not the deep vascular plexus of the skin. These findings, however, do not rule out the possibility that CLA is induced selectively in T cells located in the tissue surrounding the superficial vascular plexus. The injection of TNF-α led to the same migration rate of the CLA^+ and CLA^- HUT-78 cells, suggesting that the absence of the CLA molecule on the injected HUT-78 cells did not negatively influence the migration of these cells into the grafts. Adhesion molecules other than E-selectin were found to be upregulated in response to TNF-α injection and may have been involved in the skin migration of HUT-78 and nickel-specific T cells.

A lack of antigenic stimulation could also explain the low migration

rate of the transformed human T cells. Irani and Griffin showed in their study of T cell homing into the central nervous system that the entry of T lymphocytes was dependent upon their activation state, but their local retention and proliferation was controlled by antigen-specific reactivation (Irani and Griffin, 1996). Thus, in contrast to the nickel-reactive T cell lines, a lack of antigenic stimulation could explain the low identification rate of HUT-78 cells in the skin grafts.

Together, these findings suggest that the grafts do not accumulate HUT-78 cells in large numbers in this human skin/SCID mouse model. The lack of unequivocal morphologic identification and localization of the injected cells within the graft clearly limits the further use of the HUT-78 for T cell skin migration studies. In contrast, we induced abundant T cell migration to human skin using nickel-reactive T cell lines and nickel challenge of the grafts. Apart from selective dermal accumulation of $CD3^+$ T cells and epidermal necrosis, we did not find other hallmarks of allergic contact dermatitis such as spongiosis. A targeted search for T cell markers and activation parameters of the injected T cells was not possible because the PKH-26-labeled cells could not be double-stained with immunohistochemical methods. Petzelbauer *et al* (1996), who found increased IL-2 receptor expression on perivascular dermal graft T cells, were the first to give evidence for a dermal delayed type hypersensitivity reaction in a similar model. This study confirms that the human skin/SCID mouse model is a useful tool to elucidate the *in vivo* mechanisms of human T cell migration to skin. Our experiments with PKH-26-labeled T cells demonstrate the advantage of visualizing the migrated cells in the tissue and thus differentiating them from locally expanded T cells. Our results with the nickel-specific T cell lines also show that *in vitro* cultured human T cells may retain their ability to migrate to human skin in such a human skin/SCID mouse model. Previous experiments by Petzelbauer *et al* (1996) using tetanus toxoid were carried out with cells that were not cultured before administration to human skin/SCID mice.

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